REMARKS

Status of the Claims

Claims 10, 11, 19-24, and 26-29 are pending in the application. Claim 25 is cancelled by the present amended. Claims 10, 11, 24, 26, 28 and 29 are amended by the present amendment.

Claims 10, 11, 24, and 29 were amended to specify that the instantly claimed method is a method of treating intestinal disorders. This amendment is supported through out the specification, particularly at paragraphs [0024] and [0025].

Claim 10 was amended to specify that the instantly claimed method comprises oral administration. This amendment is supported at paragraph [0050].

Claim 10 was amended to clarify that the claimed method utilizes a recombinant microorganism that expresses a trefoil peptide in vivo. This amendment is supported throughout the specification. See, for example, paragraphs [0012]-[0014].

Claims 11, 24, and 29 were amended by deleting the word gastrointestinal to conform these claims to claim 10, which specifies intestinal disorders.

Claim 26 was amended so as to depend from claim 10, rather than now cancelled claim 25.

Claim 28 was amended to depend from claim 10, rather than from claim 27.

The present amendments do not add new matter. Applicants respectfully request that these amendments be entered and made of record in the application.

Acknowledgement of Interview with Examiner Devi

Applicants graciously thank Examiner Devi for meeting with Dr. Phillippe Jacobs and Dr. Lothar Steidler and teleconferencing with Raymond Reese on December 10, 2004. The content of the interview is briefly summarized here and specific aspects of the interview are discussed in more detail in the section of this paper addressing the rejections under 35 U.S.C. § 103.

The rejection of the instant claims under 35 U.S.C. § 103, and specifically the rejection over Podolsky and Malin, in view of Steidler was the primary topic discussed. During the interview, Applicants acknowledged that the art suggests that trefoil peptides would likely be useful for treating intestinal disorders such as colonic diseases. However, the art did not disclose that intestinal injuries could be efficiently treated via oral administration of trefoil peptides. In fact the art indicates that such peptides would be readily degraded. Further, the art did not disclose that bacteria expressing trefoil peptides would result in effective healing of intestinal injuries.

Applicants further stated that a skilled person would not consider using bacteria to deliver trefoil peptide based on the Babyatski reference (Gastroenterology, **1996** *110* 489-97), because this reference describes using 1-15 mg of trefoil peptides. To deliver this quantity of peptide using bacteria would require 2000 more bacteria than presently used in the practice of the present invention. (*See, for example*, experiment 2 of Dr. Steidler's Declaration, describing using 10 inocula of 2×10^9 CFU each). It would not be feasible to use the quantity of bacteria that would be required to deliver the amount of trefoil peptide taught by Babyatski. Likewise, it is not

presently technically realistic to engineer bacteria that express 2000 times more trefoil peptides. Consequently, a skilled artisan would not have considered using bacteria, based on Babyatski.

Applicants also explained to the Examiner that the Poulson reference teaches that trefoil peptides stick to mucus and therefore only low amounts of orally delivered trefoil peptides would transit the gut. One of skill in the art would not expect orally delivered trefoil peptides to be effective for treating intestinal disorders, based on Poulson. This is discussed in more detail below.

The Examiner was presented with the data that is being made of record in the accompanying 132 Declaration of Dr. Steidler. The content of the Declaration is discussed in more detail below. Briefly, the data show that the bacteria slide in between the gut cells where they produce bio-active trefoil peptides close to their receptor, which explains why the present invention is unexpectedly effective. In other words, the bacteria are able to circumvent the mucus barrier and to deliver the proteins where they should work.

Examiner Devi presented applicants with the claims of U.S. Patent No. 6,221,840 by Podolsky, which relates to treating gut diseases via trefoil peptides. Applicants reemphasized that the state of the art suggests that orally delivered trefoil peptide would not be effective for treating intestinal disorders.

Though no agreement was reached at the interview, the Examiner acknowledged that the present amendments and the data contained in Dr. Steidler's declaration are sufficient to overcome the rejection under 35 U.S.C. § 103, over Podolsky and Malin, in view of Steidler.

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Rejections under 35 U.S.C. § 112

Claim 25 was rejected under 35 U.S.C. § 112 as being indefinite for using the term "is is". Claim 25 has been cancelled and part of its contents (oral administration) incorporated into claim 10. The rejection of claim 25 under 35 U.S.C. § 112 is now mute.

Claim 28 was rejected under 35 U.S.C. § 112, second paragraph, as being vague, indefinite and confusing. The Examiner alleged that it is unclear whether 'a recombinant vector comprising a trefoil-coding sequence is the peptide-coding sequence, promoter sequence, the signal sequence, or an additional nucleotide sequence'. Claim 28 has been amended to depend from claim 10, rather than from claim 27. This amendment obviates the rejection under 35 U.S.C. § 112, second paragraph.

Rejections under 35 U.S.C. § 103

Rejection over Podolsky and Malin, in view of Steidler. The Examiner has maintained her rejections of claims 10, 11, 19-21, 23-25, and 27 under 35 U.S.C. § 103 as being unpatentable over Podolsky and Malin, in view of Steidler. Briefly, the Examiner alleged that Podolsky teaches the therapeutic role of pS2 (TFF1) trefoil peptide in intestinal or gastric lesions; Malin teaches the therapeutic effect of L. casei in Crohn's disease; and Steidler teaches that any biologically active peptide or polypeptide antigen can be delivered in vivo via Lactobacillus to sustain the antigen's biological activity on a mucus membrane for a sufficient length of time. The Examiner alleged that it would have been obvious to one of skill in the art to combine the teachings and arrive at the presently claimed invention. Applicants respectfully traverse.

For a combination of references to render a claim obvious, there must be a) a suggestion or motivation to combine reference teachings, b) a reasonable expectation of success, and c) the references must teach all of the claim limitations, *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Podolsky determined that trefoil peptides are naturally expressed in great abundance at the mucosal surface of the gastrointestinal tract, and are in fact expressed to a significantly greater extent in the proximity of the injured bowel. Podolsky further demonstrates (page 28) that trefoil-deficient mice that are treated with DSS (dextran sulfate sodium) develop severe colonic erosions. One of skill in the art might likely conclude, as recognized by the Examiner and acknowledged by Applicants in the present specification (see, paragraph [007]) that trefoil peptides would likely be useful for treating intestinal disorders such as colonic diseases.

However, the mere recognition that trefoil peptides might be useful for treating intestinal disorders is not sufficient to render obvious a particular delivery means of treating intestinal disorders. One of skill in the art recognizes that a reasonable expectation of successful pharmacological treatment depends on having a delivery method that is compatible both with the pharmacological agent that is to be delivered and with the target organ/area to which it is to be delivered. Podolsky does not teach any method of treatment by any means of delivery, so one of skill in the art would have to look beyond Podolsky to derive at a method of treating intestinal disorders. Apparently, the Examiner recognized this fact and has searched for additional art to construct a 35 U.S.C. 103 rejection, rather than rejecting the claims under 35 U.S.C. 102.

Malin describes the effect of oral bacteriotherapy with *Lactobacillus casei* GG in the nutritional treatment of Crohn's disease and juvenile chronic arthritis. As shown and described

therein, the use of orally administered *Lactobacillus* resulted in an increase in the guts IgA immune response, suggesting that bacteria promotes the antigen-specific IgA immune response in associate with Crohn's disease (page 143, column 2). Malin has nothing to do with using *Lactobacillus* GG to deliver pharmacologically active agents; rather, Malin is directed to using *Lactobacillus* GG as "a nutritional adjunct treatment in gastro-intestinal disorders associated with impaired mucosal barrier" (page 144, column 2). One of skill in the art would not be motivated to combine Podolsky, which teaches trefoil peptides, with Malin, which teaches bacteriotherapy and has nothing to do with drug delivery. If one did combine these references, they would arrive at a mixture of peptides and bacteria, rather than the presently claimed method of treating intestinal disorders using a recombinant microorganism that expresses a trefoil peptide *in vivo*.

Steidler describes that bacteria, such as *Lactobacteria*, can be used to deliver/administer bioactive proteins *in situ*. While Steidler does teach a delivery method for pharmacologically active proteins, there are many delivery methods known in the art. Steidler does not suggest that its method would be particularly suited for delivering trefoil peptides to the intestines. As the Examiner recognized, if Steidler did teach delivering trefoil peptides to the intestine to treat intestinal disorders, then Steidler would have been a 102 reference. Because Steidler is a 103 reference, the relevant question is whether one of skill in the art would be motivated to combine Steidler with Podolsky, with a reasonable expectation of success, to derive the instantly claimed method. Answering this question requires one to evaluate all of the art that one of skill in the art would consider at the time the invention was made. As shown below, the state of the art when

the invention was made would not suggest a reasonable expectation of success for arriving at the presently claimed method.

Based on the state of the art at the time the invention was made, one of skill in the art would not have expected the oral administration of a recombinant microorganism expressing a trefoil peptide *in vivo* to be successful for treating intestinal disorders.

In the response to the previous Office Action Applicants asked the Examiner to consider an article by Poulsen, *et al.* (Gut, 1999, Vol. 45, pp. 516-522). Poulsen details a study comparing oral and systemic pTFF2 with respect to the healing of gastric and duodenal ulcerations, and details the metabolism and distribution of the peptides in the gastrointestinal tract. While some benefit was observed in the upper gastrointestinal tract, no beneficial effect appeared in the colon. As specifically stated by Poulsen, the fact that parenteral trefoil factor 2 (pTFF2) binds to the mucus layer of the intestine and is apparently fermented and degraded in the caecum by bacteria "suggests that a beneficial effect of orally administered TFF2 in the colon is unlikely" (page, 517 and page 522, lines 11-13). In fact, duodenal ulceration was aggravated by orally administered trefoil protein. See, page 519, lines 37-44). These findings explicitly show that the suggestion of Podolsky does not appear to work in the intestine, especially below the caecum in regions such as the colon.

The Examiner dismissed Poulsen's conclusions as mere speculations. It is not speculation that 24 hours after oral administration, only 6 % of radio labeled 125I-pTFF2 is left in the gastrointestinal tract, whereas 52 % is present in the urine and 27 % in the thyroid. *See*, Poulsen, p. 522. The Examiner stated that Poulsen is specific to TFF2. The relevant question is not whether Poulsen precludes every embodiment of Applicants' invention. Rather, the question

is whether one of skill in the art would be motivated, in light of the state of the art, to invest the time, effort, and money on a quest that published data suggests is not reasonably likely to succeed.

Furthermore, Applicants demonstrated in their disclosure that mice having DSS-induced colonic inflammation responded favorably when treated according to the present invention, i.e., orally with a recombinant microorganism expressing a trefoil peptide *in vivo* (in this case TFF1), whereas mice treated orally with purified TFF1, did not respond to treatment.

The Examiner also dismissed Applicants' showings, stating that Applicants' failure to treat colitis in mice by administering TFF1 was likely due to a suboptimal dose of TFF1, the animal origin of TFF1, potential improper folding of TFF1, or lack of prolonged or repeated administration of TFF1. The Examiner did not offer any evidence to support her analysis. This unsupported speculation on the part of the Examiner does not meet the evidentiary burden for supporting a statutory rejection. *See, In re Zurko*, 258 F.3d 1379, 59 USPQ2d 1693 (Fed. Cir. 2001). If the Examiner insists on relying on such speculation to support her rejection, Applicants respectfully request that the Examiner provide supporting documentary evidence for her conclusions regarding Applicants' experimental results, as is required by Federal Circuit law. *See, id.*, 258 F.3d at 1386, 59 USPQ2d at 1697 ("[T]he Board [or examiner] must point to some concrete evidence in the record in support of these findings" to satisfy the substantial evidence test).

Not withstanding the Examiner's blanket dismissal of them, Poulsen and Applicants' own data demonstrate that it would not be expected that providing either TFF1 or TFF2 directly to the colon would be effective for treating intestinal disorders.

The Examiner also stated that Applicants' failure to treat acute colitis in mice (and by implication, Poulsen's conclusion that such a treatment would be ineffective) appears to be contrary to Playford and Chinery. In fact, neither Playford nor Chinery actually shows that trefoil proteins are effective for treating intestinal disorders, via any delivery method. Playford demonstrates that transgenic mice that overexpress human pS2 trefoil peptide have increased resistance to intestinal damage. Chinery shows that trefoil peptides protect against stomach ulcers. Neither Playford nor Chinery actually tried to treat intestinal disorders with trefoil proteins. Weighing the fact that neither Playford nor Chinery teach anything about the robustness of trefoil peptides in the intestine or the treatment of intestinal disorders by providing trefoil peptides, against the fact that Poulsen presents data that TFF2 does not survive long in the colon and Applicants own data demonstrates that purified TFF1 is ineffective for treating colitis, one of skill in the art would conclude that there would not be a reasonable expectation of success such as to render the instantly claimed method obvious.

In sum, the Examiner has not cited any suggestion in the prior art that intestinal disorders have been successfully treated by orally delivering a trefoil peptide to the intestine by any delivery means. Rather, the art, and Applicants' own experimentation, suggest that doing so would be unsuccessful. The most that the art suggested was that trefoil proteins are related to gastrointestinal inflammation.

In fact, the results provided by the presently claimed invention are quite surprising and unexpected. In view of the state of the art at the time the invention was made, one of skill in the art would have expected that if they used a recombinant bacteria as per Steidler to orally deliver the trefoil peptides of Podolsky to treat intestinal disorders, the trefoil peptides would simply

stick to the mucus in the gut and be degraded, as taught by Poulsen. Surprisingly, this does not happen. Applicants have conducted further experiments and can now explain why the present method of delivering trefoil peptides succeeds, where one of skill in the art would have expected to fail, based on the state of the art at the time the invention was made.

A previously unknown property of a recombinant microorganism expressing a trefoil peptide *in vivo* allows the microorganism to penetrate through the mucus layer and intercalate among the cells in the intestine and thereby express the peptide so that it can be taken up by the cell and not be degraded. This is a property of the bacterium itself and is not a result of the fact that the bacterium expresses TFF.

Applicants are submitting a 132 Declaration by inventor Lothar Steidler with this response. Referring to the 132 Declaration, a saturated overnight culture of IL-10 producing *Lactococcus lactis* MG1363[pT1mIL10] and control *L. lactis* MG1363 were concentrated 50 times in BM9. In 129 SvEv IL10-/- mice, isolated intestinal loops were inoculated with 100 µl of this suspension. Following loop inoculation, the mice were incubated for 30 minutes. The loops were dissected out subsequently and snap frozen in liquid nitrogen. Appropriate cryosections were prepared and stained and visualized with confocal laser scanning microscopy. Figure 1A shows a cross section of intestinal loop, overlapping with the luminal content (L) and tissue (T), both indicated by white bars. High power magnification (Fig. 1B) shows penetration of L. lactis in between the cells of the intestinal tissue. Tissues were stained with Anti-*L. lactis*, detecting *L. lactis* ; DAPI, detecting nuclei of eukaryotic cells. Cross section of intestinal loop, at the intestinal tissue shows penetration of L. lactis in between the cells of the intestinal tissue

(Fig 1C). Tissues were stained with Anti-*L.lactis* detecting *L. lactis* ; phalloidin detecting actin as present in eukaryotic cells ; DAPI : detecting nuclei of eukaryotic cells.

Fig. 1C shows that the bacteria can penetrate in between the tissue cells in the gut, and thereby produce protein in close proximity to its receptor. That is why bacteria-produced protein has a beneficial effect on inflammation in the intestine, whereas purified protein simply sticks in the mucus and is ineffective. This mode of action could not have been foreseen at the time the invention was made. One of skill in the art would not have expected bacteria-produced protein to be any more effective than purified protein. The benefits of the present invention are only understood with the hindsight knowledge of Applicants' disclosure.

In Experiment 2, ten serial inocula of 2 x 10^9 CFU of LL-pTREX1, LL-mTFF1, LL-mTFF2 or LL-mTFF3 (i.e. control *L. lactis* and *L. lactis* producing myc tagged mouse TFF1, myc tagged mouse TFF2 and myc tagged mouse TFF3 respectively, as described in Vandenbroucke *et al.* Gastroenterology, 127, 502) in 100 µL BM9 suspensions were administered to female BALB/c mice at intervals of 30 minutes. A control group received ten serial inocula of 100 µL BM9. Colons were excised one hour after the last inoculation and the entire organs, including the contents, were immediately homogenized in PBS containing 1% BSA as described below. Alternatively, contents were removed, tissues were rinsed with PBS and were immediately homogenized in PBS containing 1% BSA as described below. CFU values of the various *L. lactis* strains in the colon with contents averaged 5 x 10^8 (Table 2) and 5 x 10^6 in the colon tissue without contents (Table 1). The assay of Myc tagged TFF derived from *L. lactis* detected 8.7 ng mTFF1, 10.5 ng mTFF2 and 7.5 ng mTFF3 per total colon with contents

(Table 1) and 0.75 ng mTFF1, 0.98 ng mTFF2 and 0.83 ng mTFF3 per colon in the colon tissue without contents (Table 2).

For quantification of Myc-tagged TFF secreted *in vivo* in colon tissue, the entire colons with contents or the colon tissue without contents were homogenized in PBS containing 1% BSA and were subsequently sonicated. Myc-tagged TFF were captured from the suspension by immobilized polyclonal rabbit anti-Myc Ab (MBL, Naka-ku Naoya, Japan), and quantified by anti-Myc biotin conjugated mouse mAb (MBL) and revealed with horseradish peroxidase-conjugated streptavidin and reaction with TMB substrate (Pharmingen, San Diego, CA).

Experiment 3 describes additional studies that were conducted using a microorganism that expresses IL-10. These studies show that the microorganism does not express the protein in the bulk compartment of the various regions of the intestine; as would have been expected in view of Steidler. Rather, they penetrate in between the intestinal cells and produce the protein there. In Experiment 2, a saturated overnight culture of IL-10 producing *Lactococcus lactis* MG1363[pT1mIL10] and control *L. lactis* MG1363 were concentrated 50 times in BM9. IL-10 knockout mice (129 SvEv IL10-/-) were inoculated two by two intra-gastricly with 100 µl of the respective bacterial suspensions. The mice were sacrificed at time points 0, 30 min, 1, 2.5 and 5 hrs after inoculation. The gastrointestinal tract was removed and indicated compartments were dissected out. The contents were collected and the intestinal tissues were washed twice with phosphorous buffered saline solution (PBS). The tissues were put in 1 ml PBS p 7.4 and homogenized. The number of colony forming units (cfu), representing the number of *L. lactis* present, was determined for both the content and the homogenized tissue of each compartment

by plating on solid agar plates. The amount of mIL10 was determined in the content and in the tissue of each compartment by ELISA.

Figs. 2A and 2B show that high amounts of both *Lactococcus lactis* MG1363[pT1mIL10] and control *L. lactis* MG1363 are present in the lumen. Fig. 2C shows that mIL-10 is not detectable in any of the compartments other than the stomach. In contrast, mIL-10 is detectible in the tissue further down in the caecum and colon (see Fig. 2D). This data shows that even though mIL-10 does not survive in the lumen, the transformed bacteria produces mIL-10 in a way such that the IL-10 is able to partition into the lumen tissue.

For a further set of trials an overnight grown culture was concentrated 50 times in BM9. Four 129 SvEv IL10-/- mice were inoculated intra-gastricly with 100 µl of this MG1363[pT1mIL10] suspension. Two mice were killed 2.5 hrs after inoculation and the remaining two mice 5 hrs after inoculation when the bacteria had reached the caecum and colon. The caecum and colon were removed. The content was isolated and the tissue was washed twice as in procedure 1. The tissue was homogenized in 1 ml PBS pH 7.4. The cfu count and the amount of mIL-10 were determined in content and the tissue. Fig. 3 shows that these compartments contain substantial amounts of bacteria, but no mIL-10, whereas the tissues contain much less bacteria but high amounts of mIL-10. As in the previous experiment, the bacteria produces mIL-10 in such a way that the mIL-10 partitions into the tissue, even though the protein does not survive in the content of the lumen.

The experiments described in Dr Steidler's 132 Declaration support Applicants' assertion that a previously unknown property of the microorganisms used in the instantly claimed methods yield results that could not have been predicted based on the art available to one of ordinary skill

at the time of the invention was made. It is only through the hindsight of Applicants' invention that these results are realized.

Rejection over Podolsky in view of Le Page, Wells, and Wells. The Examiner maintained her rejection of claims 10, 11, 19-25 and 27 as being unpatentable under 35 U.S.C. § 103(a) as being unpatentable over Podolsky, in view of Le Page (WO 93/17117), Wells (Mol. Microbiol. 8:1155-1162, June 1993) and Wells (Appl. Environ. Microbiol. 59:3954-59). Briefly, the Examiner alleged that Podolsky teaches the therapeutic role of pS2 (TFF1) trefoil peptide in intestinal or gastric lesions; Le Page demonstrates the use of food-grade organisms for the recombinant delivery of peptides *via* a variety of routes. Further according to the Examiner, the Wells Article 2 teaches the use of recombinant *Lactococcus* strains for the expression of a heterologous protein using appropriate expression-secretion vectors, while the Wells Article 1 demonstrated that a heterologous peptide antigen could be expressed in substantial quantities and in soluble form via the expression of a food-grade bacteria. Consequently, and according to the Examiner, it would have been *prima facie* obvious to one of skill in the art at the time of the invention to express the Podolsky trefoil peptide recombinantly in Le Page's or Wells' (Article 1 or 2) *Lactobacillus lactis* to produce the Applicants instant invention.

As pointed out above, Podolsky suggests only that trefoil proteins would likely be useful for treating intestinal disorders such as colonic diseases. Podolsky does not suggest any means of delivering such proteins in a way that one of skill in the art would expect to be effective, based on the state of the art when the invention was made. Le Page and the Wells articles do not teach any delivery means that one of skill in the art would expect to be successful, given the state of the art at the time the invention was made. Specifically, these articles do not suggest any

delivery means that would be expected to overcome the known barriers to treating intestinal disorders with trefoil proteins, as described above. Applicants therefore respectfully request that this rejection under 35 U.S.C. § 103 be withdrawn.

Rejection over Podolsky, Malin, Steidler, and Silk. Claim 26 was rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Podolsky as modified by Malin and Steidler as applied to claim 10, and further in view of International Patent Application No. WO 8203329 to Silk, *et al.* (hereinafter, "Silk"). According to the Examiner, it would have been obvious to one of skill in the art to use Silk's gastric catheter to deliver Podolsky's proposed therapeutic composition as modified by Malin and Steidler to produce the instant invention.

One of skill in the art would not expect that using a gastric catheter, as per Silk, would overcome the recognized barriers to using trefoil peptides for treating intestinal disorders, as described above. Applicants therefore respectfully request that this rejection be withdrawn.

The Examiner is encouraged to call the undersigned should any further action be required for allowance.

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